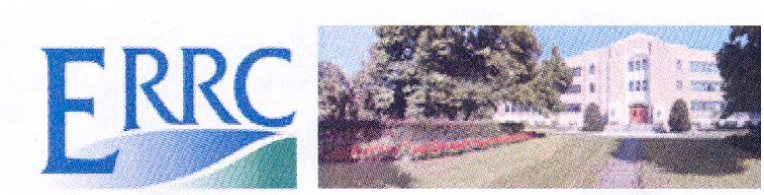

EASTERN REGIONAL RESEARCH CENTER
AGRICULTURAL RESEARCH SERVICE
UNITED STATES DEPARTMENT OF AGRICULTURE
600 E. MERMAID LANE
WYNDMOOR, PA 19038
(215) 233-6400

Title: Rapid Detection of Outbreak *Escherichia coli* O157 and *Salmonella* on Alfalfa Sprouts by Immunomagnetic Capture and Time-Resolved Fluorescence

Author(s): Shu-I Tu, M.H. Golden, W.F. Fett, A.G. Gehring and P.L. Irwin

Citation: Journal of Food Science (2003) 23:75-89

Number: 7260

RAPID DETECTION OF OUTBREAK *ESCHERICHIA COLI* O157 AND *SALMONELLA* ON ALFALFA SPROUTS BY IMMUNOMAGNETIC CAPTURE AND TIME-RESOLVED FLUORESCENCE[1]

SHU-I TU[2], MARSHA GOLDEN, WILLIAM F. FETT, ANDREW GEHRING and PETER IRWIN

USDA, ARS, NAA
Eastern Regional Research Center
600 E. Mermaid Lane
Wyndmoor, PA 19038

Accepted for Publication March 14, 2003

ABSTRACT

Commercially available alfalfa seeds were inoculated with low levels (~ 4 CFU/g) of pathogenic bacteria and sprouted at 25C. At 48 h, the spent irrigation water and sprouts were separately transferred to brain heart infusion (BHI) broth and enriched for 4 h at 37C and 160 rpm. Specific immunomagnetic beads (IMB) were then applied to capture the E. coli *O157 or* Salmonella *in the enriched media. Separation and concentration of captured pathogens were achieved using magnetic particle concentrators (MPC). IMB captured* E. coli *O157:H7 and* Salmonella *spp. then formed sandwiched complexes with europium (Eu) labeled anti-*E. coli *O157 antibodies and samarium (Sm) labeled anti-*Salmonella *antibodies, respectively. After washing the complexes, the lanthanide labels were extracted out from the complexes by specific chelators to form strongly fluorescent Eu- and Sm-chelates. The specific time-resolved fluorescence (TRF) associated with Eu or Sm was measured to estimate the extent of capture of the* E. coli *O157 and* Salmonella, *respectively. The results indicated that the approach could detect* E. coli *O157 and many* Salmonella *spp. from spent irrigation water or sprouts grown from contaminated seeds. Nontargeted bacteria, e.g., native microflora present on the untreated seeds and inoculated* Aeromonas *and* Citrobacter, *exhibited no cross-reactivity and counts were not significantly different from background fluorescence of the IMB alone. Since pathogen detection was achieved within 6 h, the assay could detect contamination levels as low as 4 CFU/g of seeds and it showed no*

[1] Mention of brand or firm names does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

[2]Corresponding Author. USDA, ARS, NAA, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19038. TEL: (215) 233-6611; FAX: (215) 233-6581; EMAIL: stu@arserrc.gov

cross-reactivity with nonpathogenic microflora present on the sprouts, the developed methodology could be used as a rapid, sensitive and specific screening process for E. coli *O157 and* Salmonella *spp. in sprouts and their irrigation water.*

INTRODUCTION

The inclusion of uncooked sprouted food products as a salad ingredient has gained considerable acceptance by American consumers. However, recent outbreaks of *E. coli* O157:H7 and *Salmonella* spp. associated with the consumption of raw sprouts have become a concern (Breuer *et al.* 2001; Brooks *et al.* 2001; CDC 2001; Feng 1997; Health and Human Services 1999; Inami and Moler 1999; Ito *et al.* 1998; Mahon *et al.* 1997; Mohle-Boetani *et al.* 2001; Proctor *et al.* 2001; Taormina *et al.* 1999; Van Beneden *et al.* 1999). If pathogenic bacteria are present on the seeds, the warm, moist, nutrient rich sprouting conditions encourage rapid growth of the pathogenic organisms to levels as high as 10^6-10^8 CFU/g of product (Andrews *et al.* 1982; Hara-Kudo *et al.* 1997; National Advisory Committee on Microbiological Criteria for Foods 1999; Splittstoesser *et al.* 1983; Stewart *et al.* 2001a). The internalization of the bacteria pathogenic to humans into the edible parts of the spouts, the cotyledons and hypocotyls, makes them difficult to disinfect after sprouting (Gandhi *et al.* 2001; Hara-Kudo *et al.* 1997; Ito *et al.* 1998).

In 1999, the U. S. Food and Drug Administration (FDA) recommended that sprout growers decontaminate seeds to reduce the microbial hazards of sprouts (FDA 1999). While currently approved treatments of seeds may reduce 99 to 99.9% of microbial populations, they do not guarantee a pathogen-free sprouted product (Beuchat 1997; Brooks *et al.* 2001; Jaquette *et al.* 1996; National Advisory Committee on Microbiological Criteria for Foods 1999; Pandrangi *et al.* 2003; Proctor *et al.* 2001; Stewart *et al.* 2001b; Taormina and Beuchat 1999a, b; Weissinger and Beuchat 2000). Testing of contaminated seeds is problematic due to low levels of seed contamination and unevenness of distribution of pathogens (Splittstoesser *et al.* 1983).

The FDA has recommended testing spent irrigation water from sprout production for the presence of *Escherichia coli* O157:H7 and *Salmonella spp.* (FDA 1999). Testing spent irrigation water has many advantages over testing sprouts. In order to test the sprouts, multiple samples must be taken from various areas of the sprouting drum to ensure that the sampling is representative of the microflora present. Also, pummeling of the sprouts to break them open prior to testing, as recommended by the FDA (FDA 1999), may release phytoalexins inhibitory to the growth of some pathogens during enrichment or isolation (Jaquette *et al.* 1996). The only disadvantage of testing spent irrigation water is that the level of microorganisms recovered is generally one log less than the level in the sprouts and low levels of pathogens may be missed (Fu *et al.* 2001). However, if the

testing of spent irrigation water is conducted at 48 h after the commencement of sprouting, as recommended by the FDA, the level of pathogens present in the irrigation water will be at a maximum level (Fu *et al.* 2001; Splittstoesser *et al.* 1983; Stewart *et al.* 2001a, b) and may be more readily detectable.

For testing irrigation water, the FDA has recommended (FDA 1999) VIP EHEC (Biocontrol Systems, Bellview, WA) or Reveal *E. coli* O157:H7 tests (Neogen Corp., Lansing, MI) for the detection of *E. coli* O157:H7 and Assurance Gold *Salmonella* EIA or Visual Immunoprecipitate (VIP) assay for *Salmonella* (both from Biocontrol Systems, Inc., Bellview, WA) for the detection of *Salmonella spp.* However, the *E. coli* tests require an overnight incubation in modified buffered peptone water with three added antibiotics and the *Salmonella* methodology requires preenrichment and enrichment for approximately 48-50 h before testing. Thus, there is a need to develop sensitive and specific alternatives that can be completed in shorter time periods.

In the past few years, we have incorporated immunomagnetic bead separation and concentration in developing new detection processes for *Escherichia coli* O157:H7 including digital fluorescent imaging of intact cells (Tu *et al.* 1998), metabolically regulated ATP bioluminescence of viable cells (Tu *et al.* 1999) and light addressable potentiometric sensor for general applications (Tu *et al.* 2000). Recently, we have combined the IMB technique with time-resolved fluorescence (TRF) of lanthanide cations, to detect *E. coli* O157:H7 and *Salmonella* in ground meats (Tu *et al.* 2001, 2002). The long fluorescence half-lives (~ 50 to 1000 μsec) and considerable Stoke's shifts (>200 nm) between the absorption and emission maxima of La cations minimize both the background fluorescence interference and random scattering of excitation light. Consequently, utilizing pulsed excitation, the fluorescence of La may be easily filtered out from the interference fluorescence and scattered excitation light by delaying the emission measurement.

A combination of time delayed fluorescence and the immunoassay has led to the development of a technique in which antibodies used to bind target species are modified to contain binding groups that form very low fluorescence La-complexes. The antibody-bound La cations are then extracted out by an "enhancement solution" that contains chelators that form strongly fluorescent products. The general principles and practical advantages of combining IMB capture and this TRF procedure have been described in our previous reports (Tu *et al.* 2001, 2002; Yu *et al.* 2002). The process is capable of detecting very low levels of *E. coli* O157:H7 and *Salmonella* spiked in ground meats and in apple cider after a short enrichment time and has a minimal cross-reactivity with nontarget bacteria that are present in high numbers in ground meats. This high degree of selectivity is important because sprouting conditions are conducive to rapid growth of indigenous microflora (Andrews *et al.* 1982; Fu *et al.* 2001; Splittstoesser *et al.* 1983) that can interfere with the growth and detection of pathogens. In the current study, we have modified and extended the developed procedure to detect

E. coli O157 and *Salmonella* in both 48 h spent alfalfa sprout irrigation water and germinated alfalfa sprouts. The results demonstrated that selected sprouted food outbreak strains of *E. coli* O157:H7, *E. coli* O157:NM and *Salmonella* were detectable utilizing our IMB-TRF developed methodology in both the spent irrigation water and sprouts germinated from contaminated seeds (~ 4 CFU/g) after a brief enrichment for 4 h at 37C.

MATERIALS AND METHODS

Bacterial Cultures

E. coli O157:H7 strain F4546, a clinical isolate associated with an alfalfa sprout-related outbreak in Michigan and Virginia in 1997 (Breuer *et al.* 2001), was obtained from Dr. Robert Buchanan, FDA, CFSAN, Washington, D.C. *E. coli* O157:NM strain 98A0626, a clinical isolate associated with an alfalfa sprout-related outbreak in 1998, was obtained from S. Abbott, California Department of Health Services (Mohle-Boetani *et al.* 2001). *Salmonella enterica* serovar Anatum strain F4317 (Pezzino *et al.* 1998), serovar Infantis strain F4319 (Pezzino *et al.* 1998), serovar Newport strain H1275 (Van Beneden *et al.* 1999) and serovar Stanley H0558 (Mahon *et al.* 1997) were all associated with alfalfa sprout-associated outbreaks and were all obtained from Dr. Patricia Griffin, CDC, Atlanta, GA. *Salmonella enterica* serovar Muenchen strain HERV2C was isolated from a seed lot implicated in an alfalfa sprout-related outbreak in 1995 (Proctor *et al.* 2001) and *Salmonella enterica* serovar Bredeney strain 3VIPHE was isolated from the waste stream at an alfalfa seed cleaning facility. Both of these *Salmonella* were isolated by one of the authors (W.F. Fett). All pathogens were stored at -20C, cultured in BHI broth and held at 4C prior to overnight inoculations. Cultures of *Aeromonas hydrophila* ATCC 7965 and *Citrobacter freundii* ATCC 8090 used for cross-reactivity testing were obtained from the American Type Culture Collection (ATCC, Manassas, VA).

Quantification of Native Microflora Present on Uninoculated Seeds and Inoculation of Pathogens Onto Seeds

Twenty-five grams of alfalfa seeds (Sprout People, Viroqua, WI) that were unwashed and had not been treated with chemical disinfectants were aseptically weighed and distributed into sterile one quart size Mason jars (Sprout People, Viroqua, WI). The total aerobic plate counts (APC) of nonpathogenic native microflora present on the uninoculated alfalfa seed was determined from twenty 25 g seed samples, pummeled in a stomacher and plated in duplicate on tryptic soy agar (TSA). The APC averaged 3.5 X 10^4 CFU/g of seeds.

The 100 mL sterile tap water (chlorine not inactivated) was added to duplicate mason jars containing 25 g of seeds for the uninoculated controls. *E. coli* and *Salmonella* cultures that were grown separately for 18 h in BHI (DIFCO, Detroit, MI) broth at 37C and 160 rpm were quantified and serially diluted in sterile tap water to 10^0 CFU/mL. One hundred mL of either *E. coli* O157 or *Salmonella* inoculated tap water was then added to duplicate mason jars containing 25 g of seed each to yield a final inoculum level of 4 CFU/g of seeds. All inoculated and uninoculated (control) jars were then closed with plastic mesh lids (Sprout People, Viroqua, WI) that had been washed, rinsed in distilled water and sprayed with 70% ethanol. The jars were then stored in a closed, opaque plastic container at 25C for 3 h. After decanting the liquid, the jars were returned to the plastic container and stored at 25C overnight.

Sprouting of Seeds and Enrichment of Spent Irrigation Water and Whole Sprouts

Twenty-four hours after the seeds were inoculated, 250 mL of sterile tap water was added to each jar, the jars were swirled for 15 s, the spent irrigation water was decanted and the jars were returned to storage in the closed plastic container. At 48 h, 250 mL of sterile tap water was added to each jar, swirled as above to mix and 9.0 mL of this 48 h irrigation water was transferred to a 15 mL sterile conical tube containing 1.0 mL of 10X BHI. The tubes were then incubated for 4 h at 37C and 160 rpm for bacterial enrichment.

To test the sprouts for pathogens, at 48 h of germination, normal strength BHI in a volume of 250 mL was added to duplicate jars with growing sprouts. The whole sprouts plus the added media were then incubated for 4 h at 37C and 160 rpm.

Capture of *E. coli* and *Salmonella*

At the end of the enrichment, 1.0 mL of broth was removed from each tube (for irrigation water) or jar (for whole sprouts) to duplicate 1.5 mL microcentrifuge tubes containing 20 μL of either anti-*E. coli* O157 antibody-coated IMB (Dynal Biotech, Oslo, Norway), for *E. coli* controls and *E. coli* inoculated sprouts or anti-*Salmonella* antibody-coated IMB (Dynal Biotech, Oslo, Norway), for *Salmonella* controls and *Salmonella* inoculated sprouts. The tubes were then incubated at room temperature for 20 min on a Speci-Mix rocker (Barnstead/Thermolyne, Dubuque, IA) to allow formation of bacterial-bead complexes. After the incubation, the beads were concentrated utilizing a magnetic particle concentrator (MPC, Dynal, Biotech, Oslo, Norway) and washed twice with washing concentrate (PerkinElmer Wallac, Turku, Finland) containing a Tris-HCl salt solution buffered to pH 7.8, Tween 20 and a preservative, diluted according to manufacturer's directions and supplemented with an additional 0.5% (v/v) Tween 20 (SIGMA, St. Louis, MO).

Labeling of *E. coli* and *Salmonella*

After the bacteria were captured by the beads, 1.0 mL of either europium-labeled anti-*E. coli* O157 antibody or samarium-labeled anti-*Salmonella* (Kirkegaard & Perry Laboratories, Gaithersburg, MD; labeled at PerkinElmer Life Sciences, Norton, OH) diluted 1:1000 in TRF assay buffer (PerkinElmer Wallac, Turku Finland), supplemented with 0.1% (v/v) Tween 20 to minimize nonspecific binding, was added to each tube. The tubes were then vortexed to mix and incubated at room temperature for 40 min on a Speci-Mix rocker to allow formation of bead, bacteria and lanthanide-labeled antibody complexes. The beads were concentrated again and washed twice as previously described.

Detection of *E. coli* and *Salmonella*

To generate highly fluorescent europium or samarium complexes, the bead, bacteria and lanthanide-labeled antibody complexes were resuspended in 100 μL enhancement solution (PerkinElmer Wallac, Turku, Finland) containing Triton X-100, acetic acid and chelates, added to separate wells of a 96-well black COSTAR 3915 microplate (Corning-COSTAR, Acton, ME) and incubated at room temperature on a plate shaker (Barnstead/Thermolyne, Dubuque, IA) for 5 min. The dissociation enhanced fluorescence in each well was measured in counts per second (CPS) utilizing a VICTOR^2 1420 Multilabel Counter (PerkinElmer Wallac, Turku, Finland) and the manufacturer's preset settings for europium or samarium. As in our two previous studies (Tu *et al.* 2001, 2002), greater than two times the uninoculated control value was the minimum level chosen for detection and all studies were done in duplicate and repeated.

RESULTS AND DISCUSSION

Sampling Choices

Sprout production uses a process that involves germinating seeds under warm, moist conditions that favor the growth of both pathogenic and nonpathogenic microorganisms present on the seeds or in the irrigation water. Pathogens may reside on or within the sprouts or be washed away from the plants by the irrigation water and carried to other sprouts. Thus, the detection of pathogens may be performed on seeds, sprouts, and/or irrigation water. However, since the contamination level in the seeds may be low and not homogeneous within any lot of seeds, the irrigation water and the sprouts were chosen for detection method development.

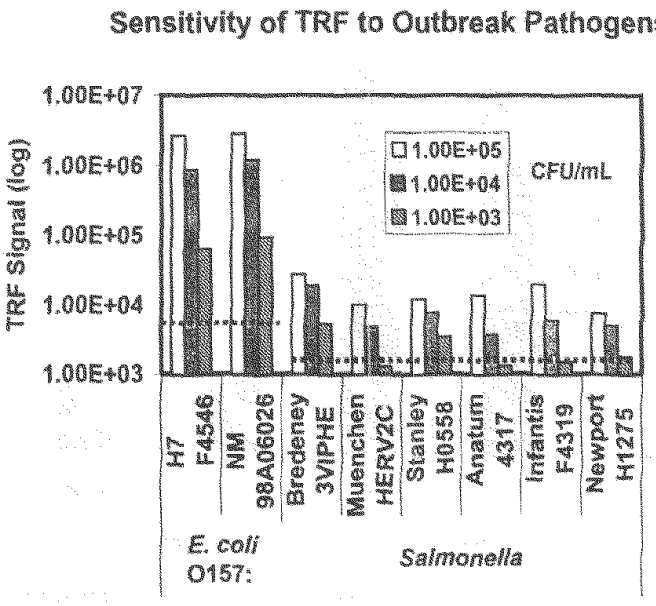

FIG. 1. SENSITIVITY OF OUTBREAK PATHOGENS TO IMB-TRF DETECTION
Outbreak strains of the *E. coli* O157 and *Salmonella* found in alfalfa sprouts were cultured in brain heart infusion (BHI) broth. The harvested pure cultures were serially diluted in BHI to prescribed concentrations. The IMB-TRF method was applied to detect the pathogens. The data shown represent averages of two independent experiments done in duplicate with probable errors equal to ± 10%. The dashed lines indicate averaged europium (for *E. coli*) and samarium (for *Salmonella*) fluorescence readings for uninoculated broth.

IMB-TRF for the Detection of Outbreak Strains of *E. coli* O157 and *Salmonella*

In our previous reports (Tu *et al.* 2002), we have demonstrated that the combination of IMB and TRF will detect both *E. coli* O157:H7 and *Salmonella* spiked in ground meats with inoculum levels as low as 10^0 CFU/g and that non-targeted microorganisms present in the meat will not interfere with the reactions. In this study, we tested this approach for its applicability in detecting similar pathogens in sprouts. First we tested our developed methodology against sprout-related outbreak strains of *E. coli* O157 and *Salmonella* to determine the sensitivity of the assay. The results are shown in Fig. 1. The *E. coli* O157 strains were detectable at 10^3 CFU/mL and the *Salmonella* strains were detectable at levels of 10^3-10^4 CFU/mL in broth culture. Both levels should be easily achievable in contaminated sprouts and their irrigation water after a 4.0 h enrichment period.

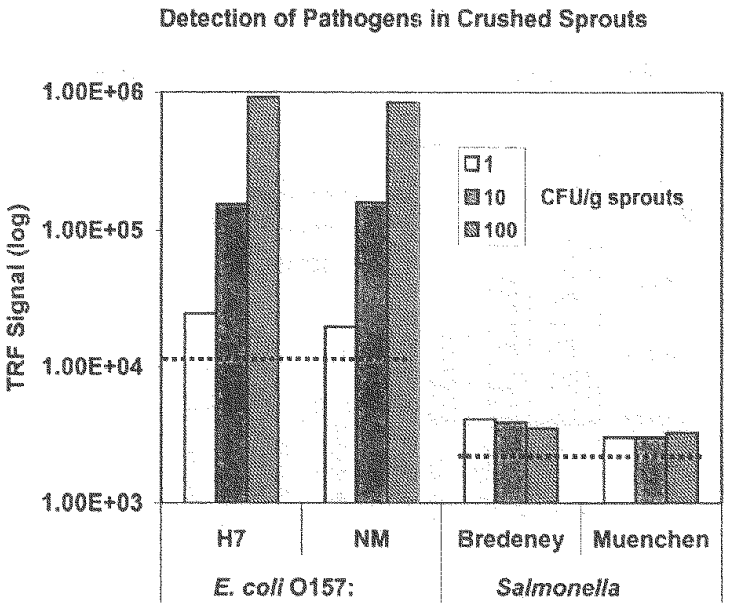

FIG. 2. SPIKING OF PATHOGENS TO COMMERCIALLY PURCHASED ALFALFA SPROUTS

Fresh sprouts were purchased from local markets and then spiked with indicated levels of pathogens. The contaminated sprouts were transferred to stomacher bags and crushed in the culture medium. After a brief incubation for 4.5 h at 37C, the pathogens in filtered suspension were tested. The data shown represent average of two experiments done in duplicate with probable errors equal to ± 10%. The dashed lines indicate averaged europium (for *E. coli*) and samarium (for *Salmonella*) fluorescence readings for uninoculated sprouts.

Detection of Pathogens on Inoculated Sprouts Obtained From a Commercial Source

We initially tested the detection method using fresh sprouts purchased from local supermarkets. Twenty-five grams of the purchased alfalfa sprouts were aseptically placed in sterile WhirlPak stomacher bags with filters, spiked with known concentrations of *E. coli* O157 or *Salmonella* diluted in 100 mL of BHI and pummeled in a Seward 400C Stomacher Lab Blender (Brinkman Instruments, Westbury, NY) at normal speed for 60 s as recommended by the FDA (1999) to release pathogens attached to the sprouts or internalized. After a brief 4 h enrichment period, 1.0 mL samples of the filtrate from the bags were removed and treated with IMB to capture targeted pathogens and then subsequently for TRF measurements as stated in Materials and Methods. The results are shown in Fig. 2. In this case, *E. coli* O157 spiked at 10^0 CFU/g of purchased sprouts, could be detected using our developed IMB-TRF methodology. However, the procedure failed to detect *Salmonella* spiked in sprouts. The basis for this has yet to be

determined but it has been reported by Castro-Rosas and Escartín (2000) that *Vibrio cholerae* O1 and *Salmonella typhi* showed no growth when inoculated onto alfalfa sprouts 24 h after germination. They attributed this observation to the abundance of competing background microflora at 24 h into the germination process. Although IMB-TRF methodology has better selectivity and higher sensitivity than the standard enrichment and culture methods employed by Castro-Rosas and Escartín , the abundance of nonpathogenic microflora, which may be as high as 9 $\log_{10}$ CFU of APC/g (Castro-Rosas and Escartín 2000), on the uninoculated commercially purchased sprouts may have impeded the growth of the inoculated *Salmonella* and, to a lesser degree, the *E. coli* O157 during enrichment. Inoculating the seeds prior to sprouting, which more closely approximates natural contamination, may enable *Salmonella* and *E. coli* O157 to successfully compete with indigenous microflora already present on the seeds.

Detection of Pathogens in Laboratory Cultivated Sprouts Grown From Inoculated Seeds

To test our system in laboratory sprouted food products, outbreak strains of *E. coli* O157 and *Salmonella* were cultured in our lab overnight. Then either *E. coli* O157 or *Salmonella* was inoculated at 4 CFU/g onto 25 g of alfalfa seeds, allowed to multiply on the sprouting plants, captured from 48 h irrigation water or sprouts by IMB and detected by TRF. Uninoculated seeds with approximately 3.5 X 10^4 CFU/g of native microflora were sprouted under the same conditions and served as controls.

The sprouts germinated in our lab were grown in sterile Mason jars and sterile tap water without the chlorine inactivated was used for irrigation. Thus, an analysis of spent irrigation water should indicate whether the sprouts, and therefore the seeds, are contaminated by pathogens. The alfalfa seeds that had been artificially contaminated with *E. coli* O157 or *Salmonella* were used to produce sprouts as described in Materials and Methods. At 48 h, nine mL spent irrigation water was removed from each sample, mixed with 1.0 mL of 10X BHI, and then shaken at 160 rpm and incubated at 37C for 4 h. After this brief enrichment, pathogen detection was performed using described IMB-TRF technology.

As shown in Fig. 3, utilizing 48 h spent irrigation water as recommended by the FDA (1999), the presence of *E. coli* O157 or *Salmonella* in alfalfa sprouts germinated from seeds with contaminated levels as low as 4 CFU/g is easily detectable. Since our previous study on ground meats demonstrated no cross reactivity between the *E. coli* O157 and *Salmonella* antibodies used in our developed procedure (Tu *et al.* 2002) the procedure may be used to both detect and differentiate *E. coli* O157 and *Salmonella* contamination of sprouted food products.

While spent irrigation water testing is convenient and is recommended by the FDA (1999), it may give false negative results because microbial counts in the irrigation water are, on the average, approximately one log lower than those seen

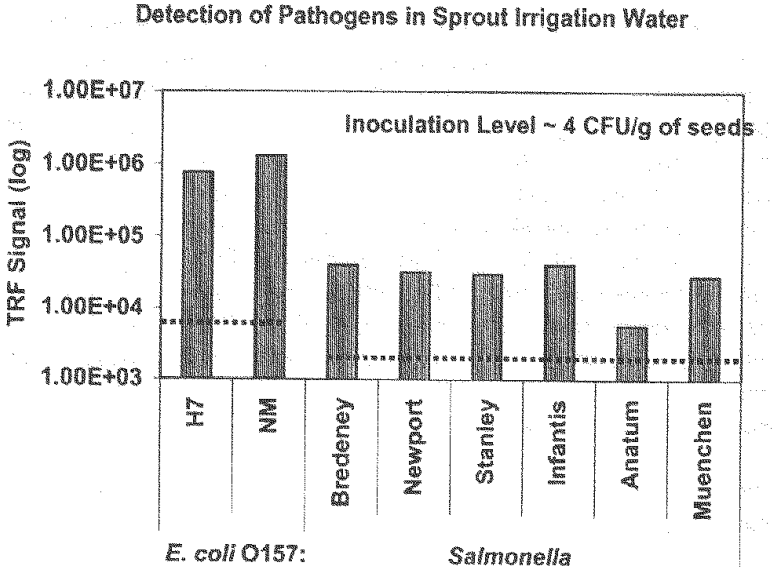

FIG. 3. DETECTION OF PATHOGENS IN IRRIGATION WATER
Commercially available alfalfa seeds were spiked with known level of pathogens (4 CFU/g). Contaminated seeds were allowed to germinate by irrigating with sterilized tap water. The water was collected and tested as described in Material and Methods. The data shown represent average of two experiments done in duplicate with probable errors equal to ± 10%. The dashed lines indicate averaged europium (for *E. coli*) and samarium (for *Salmonella*) fluorescence readings for uninoculated sprouts.

in sprout samples (FDA 1999; Fu *et al.* 2001). For this reason, we chose to apply the developed detection method to both the water and the sprouts. We did not utilize the Seward Stomacher for pummeling sprouts germinated from lab inoculated seeds because studies conducted in our lab showed lower counts for both *E. coli* O157 and *Salmonella* using pummeled sprouts as opposed to undamaged sprouts (data not shown). Instead, 25 g of whole sprouts were aseptically transferred to BHI culture medium for enrichment. With this experimental design, both the sprouts and the spent irrigation water showed the presence of the pathogens equally as depicted in Fig. 4. This experiment demonstrated that testing of 48 h spent irrigation water utilizing IMB-TRF technology will detect the presence of *E. coli* O157 or *Salmonella* on alfalfa sprouts. Unlike the results described in Fig. 2, the use of sprouts germinated from contaminated seeds under applied laboratory conditions, showed positive detection for both *E. coli* O157 and *Salmonella*. Apparently, the competitive exclusion of pathogens by background microflora is minimized by the use of pathogen-inoculated seeds and this methodology more closely approximates naturally occurring contamination of seeds.

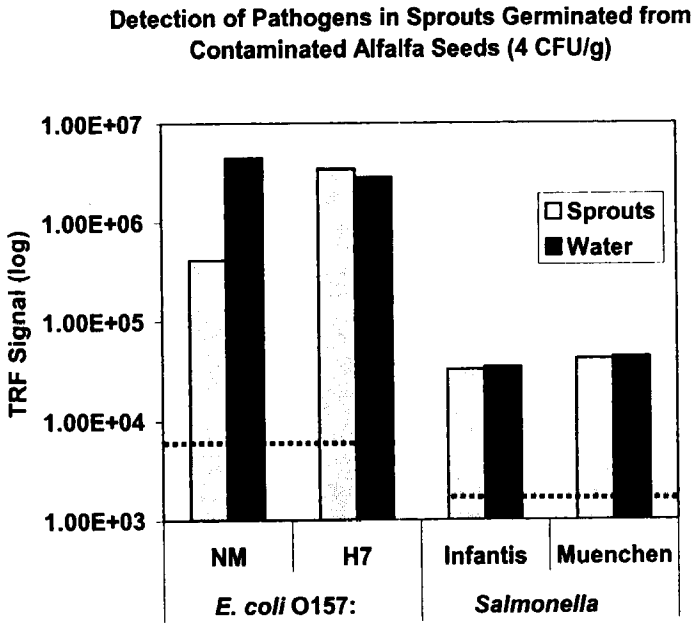

FIG. 4. DETECTION OF PATHOGENS IN SPROUTS GERMINATED FROM CONTAMINATED SEEEDS

Artificially contaminated alfalfa seeds (4 CFU/g) were used to produce sprouts. The irrigation water and the whole sprouts were separately transferred to enrichment medium as described in text. At the end of enrichment, the pathogens were detected by IMB-TRF method. The data shown represent average of two experiments with probable errors equal to ± 10%. The dashed lines indicate averaged europium (for *E. coli*) and samarium (for *Salmonella*) fluorescence readings for uninoculated sprouts.

Cross Reactivity and Specificity of the IMB-TRF Process

As described in our previous report (Tu *et al.* 2002), the developed detection method has demonstrated no cross-reactivity between *E. coli* O157 and *Salmonella* or any of the numerous spoilage organisms present in uninoculated ground beef, turkey or pork. Certain commercially available tests for the detection of *E. coli* O157 and *Salmonella* show cross-reactivity with *Aeromonas hydrophila* and/or *Citrobacter freundii* (personal communications from sprout growers). To test the developed IMB-TRF protocol for cross-reactivity against these two microorganisms, the experiment described above was repeated by replacing *E. coli* O157 and *Salmonella* with *Aeromonas hydrophila* (ATCC 7965) and *Citrobacter freundii* (ATTC 8090). Even when present at concentrations as high as $\log_6$ CFU/g, counts did not significantly differ from uninoculated controls (data not shown). Since the specificity and sensitivity remains high in our current study, the IMB-TRF pathogen detection process described in this report would be useful for the detection of both *E. coli* O157 and *Salmonella* in sprouts.

CONCLUSIONS

According to the International Sprout Growers Association (ISGA), annual human consumption of alfalfa sprouts in the United States has reached $250 million dollars (Edminster *et al.* 2001). Since 1995, raw alfalfa sprouts have emerged as a recognized source of foodborne illness in the United States. After investigation by the California Department of Health Services of sprout-related outbreaks due to *Salmonella* and *E. coli* O157, FDA issued its original Health Advisory on Sprouts in 1999. This advisory has been recently updated (10/02/2002) because of further sprout-related outbreaks of foodborne illness. The ISGA has taken positive steps to address this problem. For example, the sprout industry is pursuing the use of 2% calcium hypochlorite for soaking of the seeds prior to germination and growth. This intervention method has the potential to substantially reduce, but not necessarily eliminate, pathogenic microbial contamination of seeds that can be passed on to the consumer through ingestion of raw sprouts. Thus, it is desirable to develop effective technologies that can be applied to detect both *E. coli* O157 and *Salmonella* in alfalfa sprouts.

In our current study, we have clearly demonstrated that IMB-TRF technology has the potential to detect low levels of *E. coli* O157 and *Salmonella* in alfalfa seeds. The sensitivity of the developed immunoassay allows rapid detection of select pathogens inoculated at 4 CFU/g of seeds even when background microflora counts are as high as 3.5 X 10^4 CFU/g of seeds. The technology may be applied to both whole growing sprouts or spent irrigation water as sampling sources and the results are obtained within a time period of 6 to 7 h. Furthermore, the future development of a 96-well format would allow the developed approach to be used as a high throughput screening procedure to detect pathogens in sprouted food products.

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